Supplemental Data. Fraley et al. Cross-reactive antibody immunity in children and adults against SARS-CoV-2

Supplemental Materials and Methods

Methods

Individuals and sample collection

COVID-19 convalescent biospecimens and biospecimens from healthy adults were obtained through ProMedDx, LLC and were collected under a clinical study that has been reviewed by an Institutional/Independent Review Board (IRB) and/or Independent Ethics Committee (IEC) in accordance with requirements of local governing regulatory agencies including the Department of Health and Human Services (DHHS) and Food and Drug Administration (FDA) Codes of Federal Regulations, on the Protection of Human Subjects (45 CFR Part 46 and 2l CFR Part 56, respectively). A total of 44 individuals were diagnosed and recovered from COVID-19 were utilized in this study and a diagnosis of COVID-19 was made according to WHO interim guidance. All individuals presented as outpatients and SARS-CoV-2 infection was confirmed using quantitative PCR. The SARS-CoV-2 negative pediatric samples were collected under a research study at Children's Mercy Kansas City and both were reviewed and approved by the Children's Mercy IRB. For all biospecimens serum or plasma was utilized to perform the immunoassays that was isolated from venous whole blood collection and frozen in ultra-low temperature freezers until use.

SARS-CoV-2 viral antigen multiplexed binding assay

To measure antibody levels to SARS-CoV-2 viral antigens, S1, S2, RBD and NP we utilized a bead-based multiplex assay based on the Luminex xMAP technology using reagent kits specific for IgG (Millipore, #HC19SERG1-85Kfollowing standard protocols. In order to acquire and analyze data we utilized the Luminex analyzer (MAGPIX) and Luminex xPONENT acquisition software for data acquisition and analysis. Plasma samples were diluted 1:100. Samples were run in technical duplicate and after acquisition Net MFI was utilized which is MFI with background well MFI subtracted. Negative control beads without antigen are also run in parallel and Average MFI and standard deviation were calculated to set detection thresholds.

SARS-CoV-2 protein and peptide microarray

Plasma samples were diluted 1:200 and used to probe a single SARS-CoV-2 protein and peptide microarray (CDI Labs). After probing arrays with serum antibodies, the arrays will be washed and detected with an Alexa647-anti-human IgG Fc secondary antibody and scanned using a GenePix 4000B scanner. Array data was

collected using the MAGPIX software (Innopsys). Each protein or peptide is represented in triplicate on the microarray. There are positive control proteins (human IgG, anti-human IgG and ACE2_Fc) and blank wells to serve as negative controls. The signal intensity is measured in the detection channel 635nm (F635). The average of the F635 for each peptide was calculated and log2 transformed for graphing. Z-scores were calculated across all peptides for each individual prior to t-test analysis with Bonferroni adjustment for multiple comparisons.

Statistical analysis

All statistical analyses were performed using Prism 8.0 (GraphPad Software Inc.) software and R.

Data availability

Data that support the findings of this study are available from the corresponding author upon reasonable request.

Ethics statement

COVID-19 convalescent biospecimens and biospecimens from healthy adults were obtained through ProMedDx, LLC and were collected under a clinical study that has been reviewed by an Institutional/Independent Review Board (IRB) and/or Independent Ethics Committee (IEC) in accordance with requirements of local governing regulatory agencies including the Department of Health and Human Services (DHHS) and Food and Drug Administration (FDA) Codes of Federal Regulations, on the Protection of Human Subjects (45 CFR Part 46 and 2l CFR Part 56, respectively). ProMedDx, LLC also certifies that these biospecimens have been collected in compliance with, as applicable: i) 2l CFR 50, through obtaining informed consent from human research subjects for use of their biological materials in medical research, or ii) the FDA guidance document "Guidance on Informed Consent for In Vitro Diagnostic Device Studies Using Leftover Human Specimens that are Not Individually Identifiable" (April 25, 2006), through the biospecimens meeting all criteria of "human remnant specimens" making them exempt from the requirements of informed consent. To the extent defined above, these biospecimens meet requirements for their use in existing and future investigational research, quality control, and clinical studies to support applications for marketing permits for products, including in vitro diagnostic test kits, regulated by the FDA.

The SARS-CoV-2 negative pediatric samples were collected under a research study protocol at Children's Mercy Kansas City and reviewed and approved by the Children's Mercy IRB (Study00001265/Study00001670).

Table S1. SARS-CoV-2 Individual Metadata.

Sample ID	Age	Sex	Ethnicity	Days after infection
50153	28	F	White, Hispanic/Latino	6
51864	27	F	White, Non-Hispanic/Non-Latino	6
51883	29	M	Hispanic	80
51891	39	F	Hispanic	78
51893	50	F	Hispanic	74
51894	41	F	Hispanic	133
53186	37	F	African American	87
53263	49	M	Hispanic	74
53265	64	M	Hispanic	75
53481	28	M	White, Hispanic/Latino	6
53482	33	M	White, Hispanic/Latino	6
54876	42	F	African American	83
56088	20	M	White, Hispanic/Latino	7
56095	58	F	White, Non-Hispanic/Non-Latino	77
56147	52	M	White, Hispanic/Latino	7
56148	46	F	White, Hispanic/Latino	3
56157	26	F	White, Non-Hispanic/Non-Latino	83
56158	54	F	White, Non-Hispanic/Non-Latino	76
56159	36	F	Asian	74
201652653	59	M	African American	34
201652655	42	F	Hispanic	38
201652662	58	M	White, Hispanic/Latino	34
201652663	35	M	Asian	36
201652664	51	F	Hispanic	38
201652665	59	F	White, Hispanic/Latino	38
201652666	28	F	Hispanic	39
201652667	29	M	Hispanic	38
201652668	44	M	Hispanic	40
201652669	44	M	Other	35
201652670	42	F	Hispanic	43
201652671	39	M	Hispanic	45
201652672	30	F	Hispanic	35
201652673	33	F	African American	37
201652674	35	F	Hispanic	43

201652675	55	F	Hispanic	48
201652676	67	M	White, Non-Hispanic/Non-Latino	36
201652677	55	F	Hispanic	15
201652678	45	F	Hispanic	42
201652679	53	M	Hispanic	46
201652680	60	F	Hispanic	45
201652681	39	M	White, Non-Hispanic/Non-Latino	42
201652682	61	F	Hispanic	44
201652683	34	F	Hispanic	48
201652684	30	M	Hispanic	54

Table S2. COVID-19 Seronegative individual demographics.

	0-1 years old (N=17)	**2-10 years old** (N=37)	**11-15 years old**(N=32)	**19-39 years old** (N=44)
Gender	Male: 10 Female: 7	Male: 17 Female: 20	Male: 15 Female: 17	Male: 0 Female: 44
Race	White: 13 Black or African American: 0 Hispanic: 2 Asian: 0 Multiracial: 2 Unknown: 0	White: 12 Black or African American: 2 Hispanic: 2 Asian: 0 Multiracial: 3 Unknown: 18	White: 10 Black or African American: 4 Hispanic: 2 Asian: 1 Multiracial: 1 Unknown: 14	White: 0 Black or African American: 0 Hispanic: 0 Asian: 0 Multiracial: 0 Unknown: 44

Table S3. Significantly different peptide antibody binding between seronegative and seropositive individuals.

Peptide	p.value (t-test)	p.value.adj (Bonferroni)
S1-1	9.61E-10	2.01E-07
S1-3	2.15E-11	4.48E-09
S1-8	6.26E-05	0.01307632
S1-15	1.49E-07	3.11E-05
S1-20	5.00E-10	1.04E-07
S1-21	8.66E-07	0.00018107
S1-23	2.49E-05	0.00521142
S1-29	1.17E-07	2.45E-05
S1-33	2.00E-09	4.17E-07
S1-34	3.72E-06	0.00077786
S1-35	5.28E-11	1.10E-08
S1-37	6.23E-06	0.00130237
S1-38	0.00023594	0.04931213
S1-42	4.79E-05	0.01001632
S1-50	6.17E-05	0.01290563
S1-51	1.25E-05	0.002611
S1-56	9.96E-06	0.00208085
S1-65	4.69E-06	0.00098095
S1-66	2.44E-07	5.09E-05
S1-72	0.00013357	0.0279156
S1-82	1.67E-06	0.00034963
S1-97	0.00011494	0.02402163
S1-99	3.07E-06	0.00064212
S1-101	9.07E-06	0.00189664
S1-109	3.34E-05	0.00697204
S1-110	0.00017238	0.0360275
S1-112	1.52E-05	0.00318297
S2-10	0.00016348	0.03416696
S2-11	1.74E-05	0.00364023
S2-13	1.77E-06	0.00036964
S2-28	0.00011486	0.02400483
S2-38	8.31E-08	1.74E-05
S2-47	7.56E-05	0.01579727
S2-48	9.76E-06	0.00204041
S2-69	3.52E-05	0.00734981
S2-8	1.48E-05	0.00310133
S2-82	9.02E-05	0.01885981
S2-93	6.83E-05	0.01427558
S2-94	4.98E-05	0.01040363

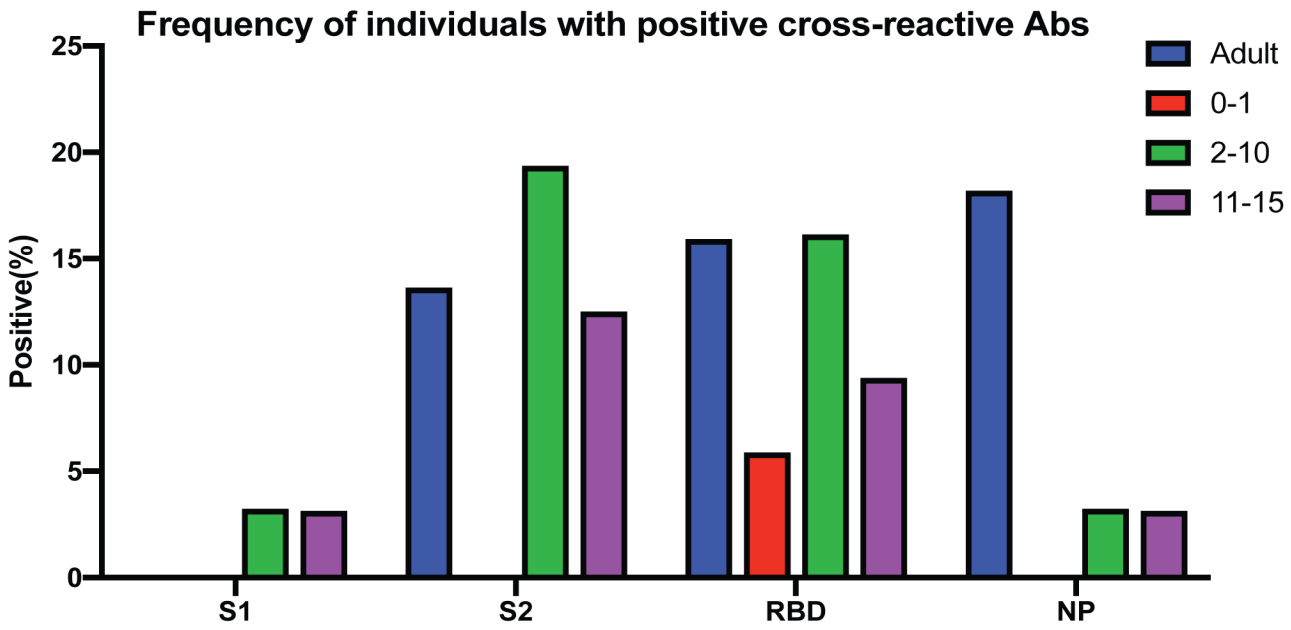

Figure S1. Frequency of positive antibody responses to SARS-CoV-2 in previously infected and healthy populations. Bar graphs showing the frequency of SARS-CoV-2 negative individuals in each age group (blue, adult; red, 0-1 years old; green, 2-10 years old; purple, 11-15 years old) that were positive for antibody using a conservative threshold of the average plus standard deviation of the whole group (adults + children) MFI values.

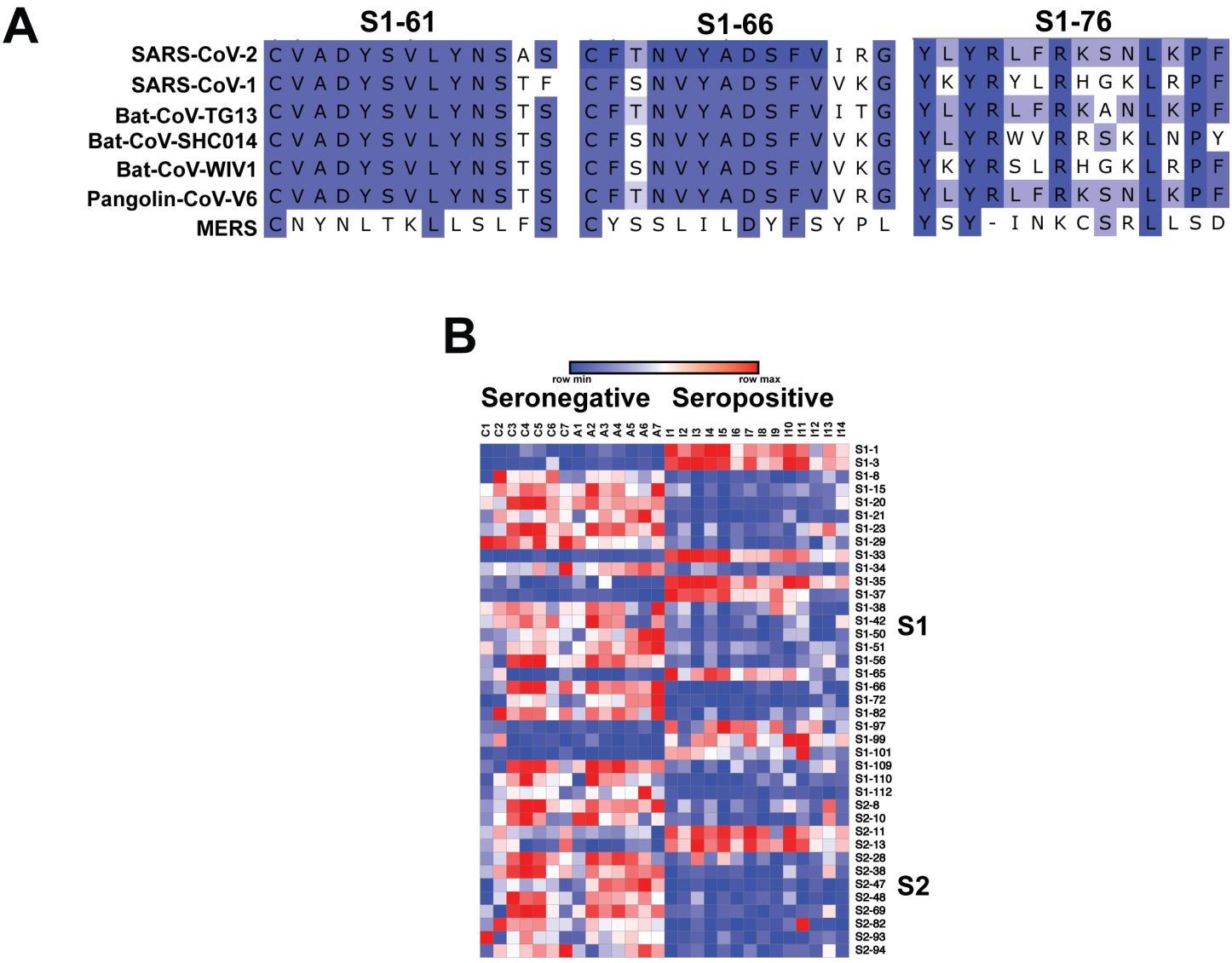

Figure S2. Immunodominant regions targeted by IgG antibodies on SARS-CoV-2 spike. (A) Amino acid sequence alignments of 3 peptides in the receptor binding domain (S1-61, S1-66 and S1-76) with related SARS-CoV-1, emergent bat and pangolin coronaviruses and middle eastern respiratory syndrome causing coronavirus (MERS). Blue shading indicates overall conservation of amino acid. **(B)** Heatmap of calculated Z-scores for peptides that have significant (Adjusted $P \leq 0.05$, t-test) differences in binding intensities between seropositive and seronegative individuals.